ELSEVIER

PEPTIDES

Peptides 20 (1999) 63–70

Comparison of rates of penetration through insect cuticle of amphiphylic analogs of insect pyrokinin neuropeptides

P. E. A. TEAL[a,*], J. A. MEREDITH[a], R. J. NACHMAN[b]

[a]*Center for Medical, Agricultural and Veterinary Entomology, USDA, ARS, PO Box 14565, Gainesville FL, 32604*
[b]*Veterinary Entomology Research Unit, FAPRL, USDA, ARS, 2881 F&B Road College Station, TX 77845*

Received 10 July 1998; Accepted 7 October 1998

Abstract

Rates of penetration through the cuticle of amphiphylic analogs, synthesized by addition of 6-phenylhexanoic acid or 9-fluoreneacetic acid or 1-pyrenebutyric acid to the amino terminus of the pentapeptide Phe-Thr-Pro-Arg-Leu-amide, were assessed by quantitative analysis using reversed phase liquid chromatography. The analogs effectively penetrated the cuticle of both the adult American cockroach and tobacco budworm moth. However, the amounts of analogs that penetrated the cuticle of the cockroach were significantly lower and the rates of penetration were slower than for moth cuticle. Penetration of the analogs through the cuticle was dependent upon the size of the lipidic attachment to the pentapeptide. The 6-phenylhexanoic acid analog penetrated most rapidly followed by the 9-fluoreneacetic acid analog and the 1-pyrenebutyric acid analog penetrated slowest. All of the analogs exhibited an initial rapid period of penetration lasting 2–3 h followed by the establishment of a steady slow release state which lasted between 9–24 h and was dependent upon both the size and surface area of the aromatic lipidic portion of the analog and species of insect to which the analog was applied. The results confirmed the hypothesis that the insect cuticle could be employed as a slow release device for delivery of analogs of insect neuropeptides. © 1999 by Elsevier Science Inc.

Insect cuticle; Neuropeptide; PBAN; Pheromone

NEUROPEPTIDES regulate virtually all aspects of insect life and are excellent candidates for development of new methods for pest control [6]. However, peptides do not penetrate the hydrophobic insect cuticle and are subjected to rapid degradation by peptidases in the gut. Therefore, commonly used methods of application of pesticides, including spray and bait station protocols, are impractical for development of control strategies employing insect neuropeptides. In order to harness the potential of insect neuropeptides for insect control we have been designing and synthesizing analogs of neuropeptides that are capable of penetrating the insect cuticle. The theory behind design of these analogs is that attachment of lipid moieties to the amino terminus of small peptides gives the molecules an amphiphylic character making them both soluble in water and capable of penetrating the hydrophobic insect cuticle [2,16,20].

Our research has centered on design of analogs that mimic the effects of the pyrokinin class of insect neuropeptides. This family of neuropeptides regulates a number of different physiological functions and is widespread among insect orders [14]. For example, among locusts and cockroaches the pyrokinins act as myotropins on the gut and oviduct [6,18,19], while in Lepidoptera they induce sex pheromone biosynthesis [8–10,17], larval reddish coloriza-

* Corresponding author. Tel: 352-374-5788; fax: 352-374-5707; e-mail: peat@nervm.nerdc.ufl.edu.

PII: S0196–9781(98)00154–5

tion and melanization [11] and in the silkworm moth they induce egg diapause [7]. Additionally, the cockroach pyrokinin, LPK, mimics the effect of proteinaceous extracts of the cephalic ganglia of the flesh fly by accelerating pupariation [14]. Although these peptides have different numbers of amino acids, they all share the common C-terminal sequence, Phe-Xxx-Pro-Arg-Leu-amide (Xxx = Gly,Ser, Thr,Val). This pentapeptide sequence forms a β-turn in the peptides and is required for bioactivity of all of the neuropeptides [12,13]. Structure-activity studies were conducted to determine if naturally occurring pyrokinins would induce sex pheromone biosynthesis in moths [1]. The results demonstrated that pyrokinins, isolated from the locust and cockroach, had a superagonistic effect because they induced production of significantly more pheromone when injected at lower doses than was produced by injection of the optimal dose of the naturally occurring pheromone biosynthesis activating neuropeptide (PBAN) [1]. These superagonists had the C-terminal sequence Phe-Thr-Pro-Arg-Leu-amide and subsequent studies indicated that this pentapeptide was as effective as PBAN in inducing pheromone production in the tobacco budworm moth [20]. Other peptides having Gly or Ser substitutions in the variable position had limited or no activity [1]. Consequently, we have used the pentapeptide, Phe-Thr-Pro-Arg-Leu-amide, as the parent peptide for synthesis of analogs capable of penetrating the cuticle of moths and inducing pheromone production.

We have succeeded in synthesizing a number of amphiphylic analogs that exhibit superagonsitic pheromonotropic effects when injected into adult moths and effectively stimulate production of pheromone when applied topically in water [2,16,20]. However, several of these analogs were active only for 4–8 h after topical application. The short periods of bioactivity limited the use of these analogs for development of control strategies because such strategies require that the analogs induce prolonged physiological responses after application. One way to increase the length of the bioactivity period is to develop slow release technologies for the analogs. We hypothesized that the chemical composition of the insect cuticle, which includes an apolar lipid layer subtended by polar protein and chitin matrices, might provide an excellent reservoir for suitably designed amphiphylic analogs and allow for slow release of the analogs. Evidence to support this hypothesis was obtained when we tested two analogs synthesized by attachment of either 9-fluoreneacetic acid (9Fla) or 1-pyrenebutyric acid (1Pba) to the amino-terminus of Phe-Thr-Pro-Arg-Leu-amide [20]. These analogs effectively induced moths to produce pheromone for periods of greater than 18 h when applied topically. Although the prolonged activity of the analogs suggested that release continued over a long period no concrete data on the release rate of these analogs was available. Additionally, we did not know if the analogs would penetrate the cuticle of other insects for which the pyrokinins are known to exert physiological effects. The following reports the results of studies in which, using reversed phase liquid chromatography, we quantified the rates of penetration of the 9Fla-FTPRLa and 1Pba-FTPRLa analogs through the cuticle of the tobacco budworm moth and American cockroach. We also compared these rates to that of an analog, formed by attachment of 6-phenylhexanoic acid (6Pha) to Phe-Thr-Pro-Arg-Leu-amide, because the 6Pha-FTPRLa analog has a bioactivity period of only 6–8 h when applied to the cuticle of moths [15].

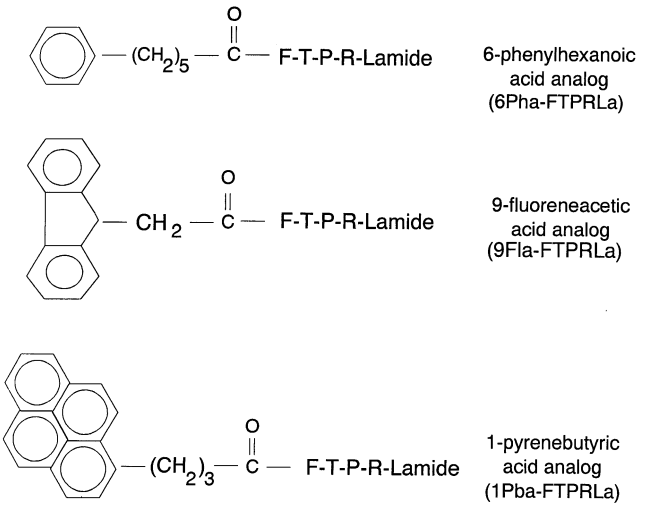

Fig. 1. Structures of analogs applied to cuticle of moths and cockroaches.

1. Methods

1.1. Pseudopeptide synthesis

The pseudopeptide analogs, 6Pha-FTPRLa, 9Fla-FTPRLa and 1Pba-FTPLa (Fig. 1) were synthesized and purified by reversed phase liquid chromatography as described previously [15,20]. Mass spectra of purified compounds were obtained using a Kratos MS-50RF mass spectrometer with an Iontech ion gun operated at an accelerating voltage of 67 Kv and with a Mack 3 data system. Quantitation was accomplished by amino acid analysis using an Applied Biosystems 420A™ amino acid analyzer after hydrolysis in 6N HCl. Structures of the analogs are shown in Fig. 1.

1.2. Cuticle preparation

Adult females of the tobacco budworm moth and American cockroach were obtained from colonies at our facility (CMAVE). We removed the scales on the surface of the abdomen of moths by gently rubbing the ventral surface of the abdomen on cellulose adhesive tape prior to dissection of the cuticle. Animals, anesthetized by submersion in H_2O for 30 min, were pinned ventral side up in a wax dissecting dish flooded with water. Lateral incisions along the margins

of the abdominal sternites were made between segments 1–7. The epidermal layers including the cuticle and epidermal cells were lifted up and associated tissue was cleared using forceps prior to removal. The epidermal tissue was placed cell side up on a microscope slide and the cells were scraped from the cuticle using a glass cover slip. To further clean the cuticle of cellular debris the tissue was floated, cell side down, in a beaker containing H_2O and subjected to sonication in a water bath at 30°C for 30 min. The cuticle strips were then washed 3 times in clean water. Pieces of cuticle, ca. 0.4 cm^2, composed of individual sternites without associated intersegmental membranes were then dissected away from remaining cuticle.

1.3. Incubation of cuticle

Incubation of cuticle was accomplished using the wells of ELISA plates (Corning, 96 well Easy Wash). Prior to use the wells of the plates were blocked to minimize adsorption of analogs to the wells by filling with a 1% gelatin in 10 mM sodium phosphate buffer containing 150 mM NaCl (pH 7.25) (PBS) and incubating at 35°C for 1.5 h. After blocking, the plates were washed with PBS-Tween and followed by three washes with H_20. The wells of the plates were filled with 350 μL of H_20 and pieces of cuticle were floated, cell side down, in the wells. Either 6Pha-FTPRLa or 9Fla-FTPRLa or 1Pba-FTPRLa (Fig. 1) (0.5 nmol) were applied to the center of the cuticle pieces in a 0.5 μL drop of H_20 using a Hamilton 10 μL syringe fitted with a fused silica needle (0.17 mm OD) and held in a Brinkmann micromanipulator. All applications and transfers were made using a microscope and the preparations were observed for 5 min after application of the analogs to ensure that drops did not slide off the cuticle. Data from wells in which the drops slid off the cuticle were not considered for analysis. Lids were applied to the plates after the drops of water containing the analogs dried (ca. 5 min after application) and the plates were placed on an orbital shaker operated at 80 r.p.m. At this speed no water contacted the upper surface of the cuticle. One h after application of the analogs we carefully transferred the cuticle to new wells containing 300 μL of H_20 and incubation was continued. Cuticle was subsequently transferred to new wells at 3, 5, 7, 9 and 24 h after application of the analogs unless stated otherwise. After incubation, 100 pmol of internal standard (9Fla-FTPRLa used when 1Pba-FTPRLa was applied to the cuticle and 1Pba-FTPRLa used when 6Pha-FTPRLa or 9Fla-FTPRLa was applied) were added to the wells. The contents of the wells were mixed by pumping the contents in and out of a micro pipette several times and then transferred to a 1.5 mL Microfuge tube. The wells were extracted two additional times by addition of 350 μL of water and the extracts combined prior to concentration to apparent dryness using a Speed-Vac concentrator.

Dried samples were reconstituted in 35% acetonitrile (MeCN) (Burdick and Jackson) prior to analysis. Reversed phase liquid chromatographic analysis (RPLC) was accomplished using a LDC Biochrome quaternary gradient pump and LDC Spectro Monitor 3200 UV detector set at 210 nm and interfaced to a Nelson Analytical 3000 data acquisition and analysis system. A Macrosphere 300 C18 reversed phase column (250 mm $\times$ 2.1 mm id, 5 μM, Alltech) was used for all separations. Solvents used for all separations were H_2O and MeCN each containing 0.1% TFA as the ion pair reagent. Samples were injected onto the column using a Rheodyne 7125 injector (100 μL loop) in 35% MeCN. The column was eluted after a 5 min equilibration period using a linear gradient from 35–75% MeCN over 90 min at a flow rate of 0.25 mL/min. Retention times of 6Pha-FTPRLa, 9Fla-FTPRLa and 1Pba-FTPLRa were 23.3 ($\pm$ .15), 24.2 ($\pm$ .11) and 38.0 ($\pm$ .25) min respectively. Analysis of eqimolar amounts of analogs indicated that the compounds had different detector responses when analyzed with the UV detector set at 210 nm. Therefore, all values were corrected to reflect the differential detector response for the analogs. Data acquired from analyses were reduced and analyzed using NCSS 97 software using regression analysis and *t*-tests.

2. Results

Initially, we assessed the rates of penetration of the analogs through the cuticle of the moth, *Heliothis virescens*. Although the analogs penetrated the cuticle, the rates of penetration were significantly different (Fig. 2). The total amounts of each of the analogs recovered over time increased in a logarithmic fashion during the 24-h sampling period ($r = 0.958$ 6Pha-FTPRLa analog; $r = 0.997$ 9Fla-FTPRLa analog; $r = 0.968$ 1Pba-FTPRLa analog) (Fig. 2). The 6Pha-FTPRLa analog penetrated the cuticle much more rapidly than did either of the other analogs. During the first hour after application 290.4 ($\pm$ 30.26) pmol of this peptide penetrated the cuticle but after that the amounts recovered declined rapidly so that during the 9 to 11 h after sampling no detectable amounts of material were recovered (Fig. 2). When we analyzed samples incubated continuously for 18 or 24 h periods we recovered 347.9 ($n = 6$, $\pm$ 24.82) pmol and 343.1 ($n = 6$, $\pm$ 25.98) pmol of 6Pha-FTPRLa respectively. These amounts were no different from that recovered at 9 h after application and indicated that all of the pseudopeptide had been released by 9 h. After the first hour after application 35.2 ($\pm$ 14.6) pmol of the 9Fla-FTPRLa analog was recovered. This was significantly less than that recovered for the 6Pha-FTPRLa analog ($t = 5.23$, 10 df) but greater than the 2.1 ($\pm$ 0.5) pmol of 1Pba-FTPRLa recovered from 1 h incubations ($t = 2.52$, 10 df). Although detectable amounts of both the 9Fla-FTPRLa and 1Pba-FTPRLa analogs were recovered at each sample interval, significantly more of the 9Fla-FTPRLa analog was recovered at every sample interval (Fig. 2).

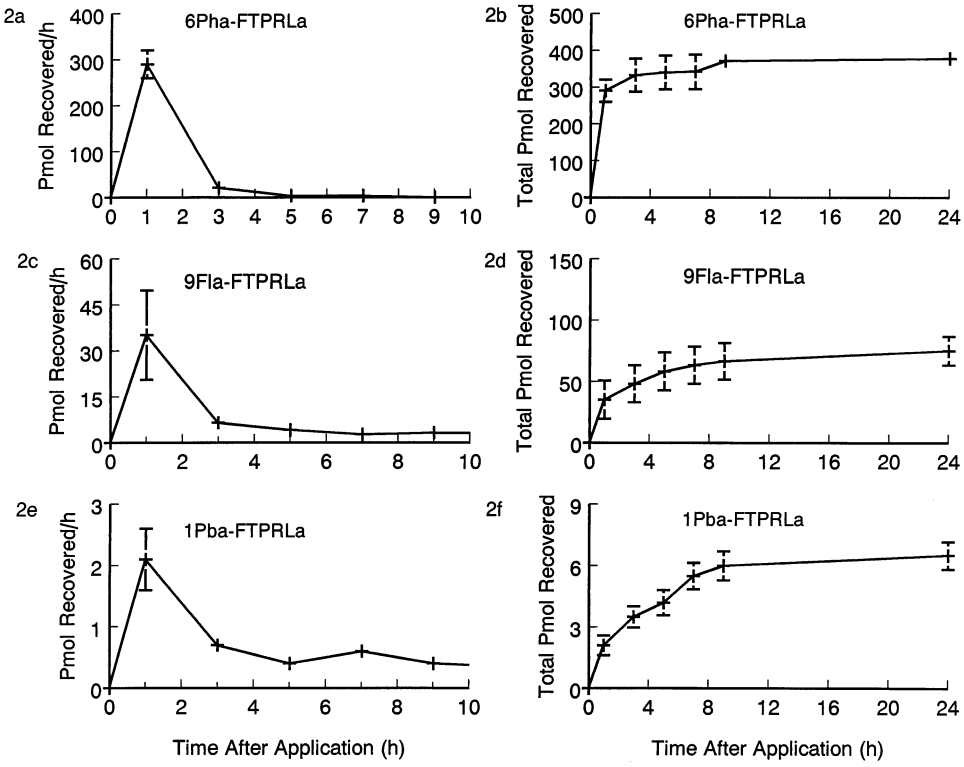

Fig. 2. Mean amounts of analogs recovered from wells of ELISA plates at intervals after application to the cuticle of moths (n = 6/replicate). (2a) Mean amounts of 6Pha-FTPRLa recovered per hour at each sampling interval; (2b) mean total amounts of 6Pha-FTPRLA recovered at each sampling interval; (2c) mean amounts of 9Fla-FTPRLa recovered per hour at each sampling interval; (2d) mean total amounts of 9Fla-FTPRLa recovered at each sampling interval; (2e) mean amounts of 1Pba-FTPRLa recovered per hour at each sampling interval; (2f) mean total amounts of 1Pba-FTPRLa recovered at each sampling interval.

Analysis of samples obtained when using cockroach cuticle indicated that the three analogs penetrated the cuticle effectively (Fig. 3). However, the amounts of the analogs that penetrated the cuticle were lower and initial rates of penetration were much slower than when moth cuticle was used. In fact, during the first hour only 16.0 (± 3.6) pmol of the 6Pha-FTPRLa analog penetrated the cuticle of the cockroach whereas 290.4 (± 30.26) pmol of this peptide penetrated the cuticle of the moth. Interestingly, the initial rate of penetration of this pseudopeptide was maintained during the first 2 sampling intervals (1 and 3 h, t = 0.57, 10df) (Fig. 3) while the amounts of both the 9Fla-FTPRLa and 1Pba-FTPRLa analogs that penetrated the cuticle during the period between 1–3 h declined significantly (t = 3.45, 10df for 9Fla-FTPRLa; t = 6.59, 10 df for 1Pba-FTPRLa). The amount of the 6Pha-FTPRLa analog recovered after 5 h was significantly lower than that recovered at the 3 h interval (t = 2.66, 10 df). Apart from these differences the curves generated from analysis of samples obtained from incubations employing cockroach cuticle exhibited the same trends as those obtained when using moth cuticle. Thus, total amounts of each of the analogs recovered over time increased in a logarithmic fashion during the 24-h sampling period (r = 0.952 6Pha-FTPRLa analog; r = 0.971 9Fla-FTPRLa analog; r = 0.963 1Pba-FTPRLa analog) (Fig. 3).

3. Discussion

The results of our study demonstrated that the three amphiphylic analogs penetrated the cuticle of both the moth, *H. virescens*, and the cockroach, *Periplaneta americana*, when applied topically in water. However, significantly less of each of the analogs penetrated the cuticle of the cockroach (Fig. 4) during the 24 h incubation period. Gravimetric analysis of the total amount of hexane soluble apolar lipid on the surface of the cuticle (cuticle extracted 3 times, 15 min each, 10 mg cuticle/ml hexane) indicated the presence of 82.3 μg (± 23.1, n = 3) lipid/mg cuticle from the cockroach and 77.0 μg (± 13.9, n = 3) lipid/mg cuticle from the moth. Therefore, differences in amounts of apolar wax on the surface of the cuticle of the two species did not account for differences in the penetration of the analogs. Thus, other structural differences between the cuticles of the

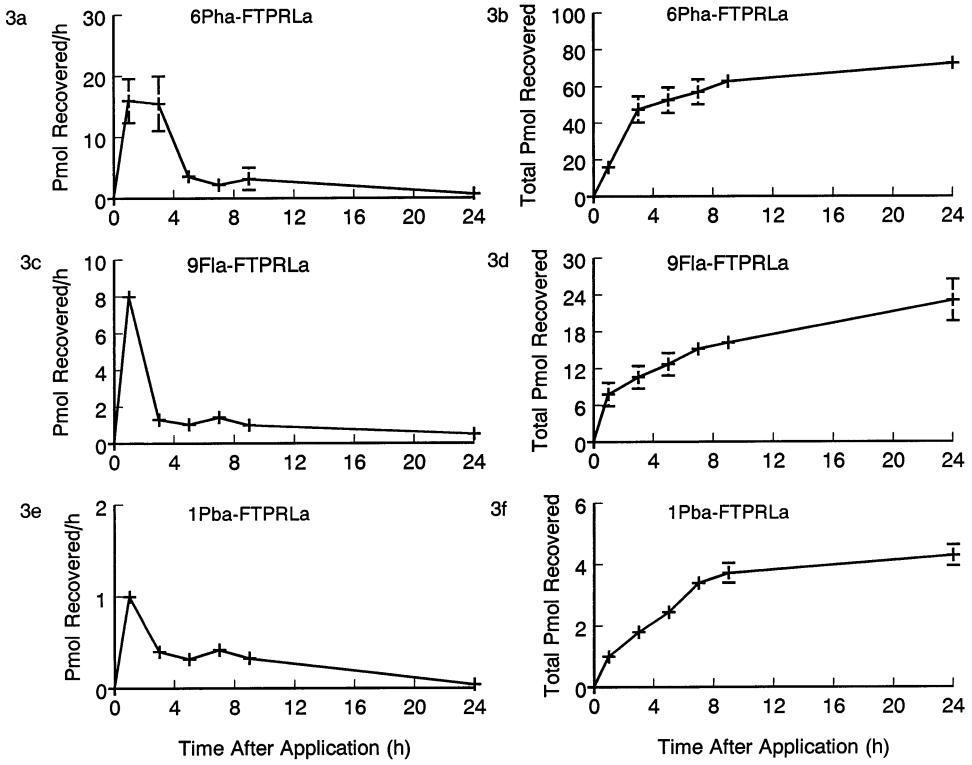

Fig. 3. Mean amounts of analogs recovered from wells of ELISA plates at intervals after application to the cuticle of cockroaches (n = 6/replicate). (3a) Mean amounts of 6Pha-FTPRLa recovered per hour at each sampling interval; (3b) mean total amounts of 6Pha-FTPRLa recovered at each sampling interval; (3c) mean amounts of 9Fla-FTPRLa recovered per hour at each sampling interval; (3d) mean total amounts of 9Fla-FTPRLa recovered at each sampling interval; (3e) mean amounts of 1Pba-FTPRLa recovered per hour at each sampling interval; (2f) mean total amounts of 1Pba-FTPRLa recovered at each sampling interval.

two species accounted for the different rates of penetration. The cuticle of the cockroach is more dense and thicker than that of the moth, which would retard penetration of the analogs. Additionally, the protein composition of the cuticular layers of the cockroach contain less than half as many polar amino acids as are present in the cuticle of other moth species [4]. Therefore, the cuticle of the cockroach is less polar than that of adult moths [4]. Consequently, the polar peptidic portion of the amphiphylic analogs might have retarded penetration through the cuticle of the cockroach relative to that of the moth.

The logarithmic rates of release of the three analogs through the cuticle of both the moth and the cockroach demonstrated that the cuticles were acting as slow release matrixes for the analogs. The initial rapid rates of release indicated that the cuticle at the region of application was saturated with pseudopeptide and that the analogs were penetrating at their maximal rates. As time progressed, analogs were absorbed and distributed more evenly in the cuticle surrounding the point of application. Therefore, equilibrium was established throughout the cuticle so that the rates of release were reduced and declined slowly in a linear fashion. Once equilibration had been established, the rates of release were equivalent for the cuticle of both species. The linear decline in release of both the 6Pha-FTPRLa and 9Fla-FTPRLa analogs occurred after 3h for both the moths (r = 0.893, 6Pha-FTPRLa; r = 0.876, 9Fla-FTPRLa) and roaches (r = 0.911, 6Pha-FTPRLa; r = 0.977, 9Fla-FTPRLa) and after 5 h for the 1Pba-FTPRLa analog (r = 0.915, moth; r = 0.952, roach).

The fact that the three analogs had different maximal rates of release could not be explained solely by differences in polarity of the analogs because the 6Pha-FTPRLa and 9Fla-FTPRLa analogs had very close retention times, 23.3 ($\pm$ 0.15) and 24.2 ($\pm$ 0.11) min respectively, when analyzed by RPLC using a C18 stationary phase. Therefore, the structure, surface area and bulk of the lipidic attachment appeared to have a significant effect on release of the analogs. The 6Pha-FTPRLa analog contains a single phenyl ring, whereas the 9Fla-FTPRLa and 1Pba-FTPRLa analogs

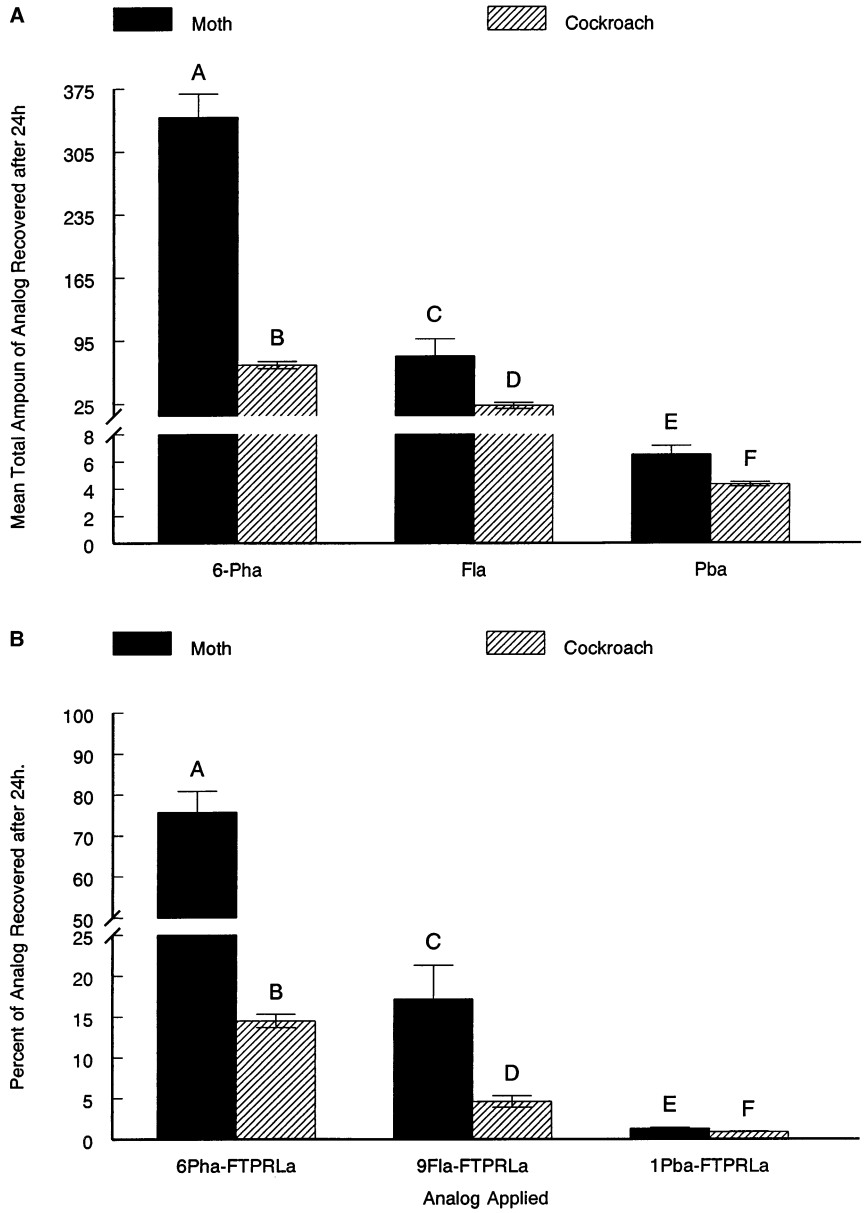

Fig. 4. Comparison of recoveries of analogs which penetrated the cuticle of the moth and cockroach (n = 6/replication/treatment). (4a) Mean total amounts of analogs recovered over 24-h. (4b) Mean percentages recovered over 24-h of the total amounts of analogs applied. Means for each pseudopeptide were different between the moth and cockroach cuticle in t-tests at $p = 0.05$.

contain multiple aromatic rings essentially in the same plane. Therefore the surface area of the B electron cloud is greater for the 9Fla-FTPRLa analog, and still greater for the 1Pba-FTPRLa analog, as compared with the 6Pha-FTPRLa analog. Different degrees of interaction between the aromatic regions of the lipid portion of the three analogs with aromatic residues in the protein component and/or other hydrophobic areas within the cuticle may influence the rates of release. The longer carbon chain of the 6Pha-FTPRLa analog may have allowed it to be absorbed into the lipid layers of the cuticle more readily than either the 9Fla-FTPRLa or 1Pba-FTPRLa analogs because it was most similar to the long chain hydrocarbons which make up the greatest portion of the cuticular lipids [3]. The 1Pba-FTPRLa analog was both the bulkiest and most apolar of the analogs and thus penetrated the cuticle much slower than the other analogs.

The fact that the rates of release of the three analogs are

dependent upon both the structures of the analogs and insect to which they are applied has significant implications for synthesis of pseudopeptide analogs designed to have prolonged effects when applied topically. The 6Pha-FTPRLa analog would have limited usefulness for inducing prolonged effects in moths because no detectable amounts of this analog were recovered after 9 h and the analog had a bioactivity period of only 6–8 h after application [15]. However, substantial amounts of the 9Fla-FTPRLa analog were released from the moth cuticle between 9–24 h after application. Based on the regression equation obtained between 7–24 h approximately 0.07 pmol would have penetrated the cuticle between 23–24 h. This quantity alone would be insufficient to induce a biologic response because significant effects were induced by injection of amounts of 0.1 pmol or greater [20]. However, the fact that this pseudopeptide resists aminopeptidase attack [20] suggests that residual amounts of the peptide still present in the hemolymph plus that expected to penetrate the cuticle between 23–24 h would be sufficient to induce a significant biologic effect. Results of bioassays support this because the amount of pheromone present in extracts obtained from glands of moths 24 h after topical application of the 9Fla-FTPRLa analog (26.4 ± 2.64 ng, $n = 10$) was significantly greater ($t = 6.54$, 18 df) than that obtained from controls in which only water was applied to the cuticle (7.89 ± 0.93 ng, $n = 10$) [20]. Thus, the use of this analog would be favored over that of the 6Pha-FTPRLa analog for application to moths. However, among cockroaches the 6Pha-FTPRLa analog would be favored over the 9Fla-FTPRLa analog because 0.13 pmol of the 6Pha-FTPRLa and 0.05 pmol of the 9Fla-FTPRLa analogs should penetrate the cuticle between 23–24 h after application. Rates of penetration of the 1Pba-FTPRLa analog were much lower than those of either of the other two analogs. Consequently, the amounts of this pseudopeptide predicted to penetrate the cuticle of the moth (0.03 pmol) or cockroach (0.02 pmol) between 23–24 h after application would probably be insufficient to induce a biologic response. Nonetheless, this analog might have considerable promise for use in organisms like dipteran or lepidopteran larvae which have much less heavily sclerotized cuticle than that of either adult moths or cockroaches.

This technique will additionally be a valuable tool in development of amphiphylic analogs of other classes of insect neuropeptides, for which practical in vivo bioassays may not have been developed.

References

[1] Abernathy RL, Nachman RJ, Teal PEA, Yanashita O, Tumlinson JH. Pheromonotropic activity of naturally occurring pyrokinin insect neuropeptides (FxPRLamide) in *Helicoverpa zea*. Peptides 1995;16:215–219.

[2] Abernathy RL, Teal PEA, Meredith JA, Nachman RJ. Induction of pheromone production in a moth by topical application of a pseudopeptide mimic of a pheromonotropic neuropeptide. Proc. Natl. Acad. Sci. USA. 1996;93:12621–12625.

[3] Blomquist GJ, Dillwith JW. Cuticular lipids. In: Kerkut GA, Gilbert LI, Eds. Comprehensive insect physiology biochemistry and pharmacology, vol. 3. New York, NY: Pergamon Press; 1985:117–154.

[4] Hepburn HR. Structure of the integument. In: Kerkut GA, Gilbert LI, Eds. Comprehensive insect physiology biochemistry and pharmacology, vol. 3. New York, NY: Pergamon Press; 1985:1–58.

[5] Holman GM, Cook BJ, Nachman RJ. Isolation, primary structure and synthesis of a blocked neuropeptide isolated from the cockroach, *Leucophaea maderae*. Comp. Biochem. Physiol. 1986;85C:219–224.

[6] Holman GM, Nachman RJ, Wright MS. Insect neuropeptides. Ann. Rev. Entomol. 1990;35:201–217.

[7] Imai K, Konno T, Nakazawa Y, Komiya T, Isobe M, Koga K, Goto T, Yaginuma T, Sakakibara K, Hasegawa K, Yamashita O. Isolation and structure of diapause hormone of the silkworm moth, *Bombyx mori*. Proc. Japan Acad. 1991;67:98–101.

[8] Kitamura A, Hagasawa H, Kataoka H, Inoue T, Matsumoto S, Ando T, Suzuki A. Amino acid sequence of pheromone-biosynthesis-activating neuropeptide (PBAN) of the silkworm, *Bombyx mori*. Biochem. Biophys. Res. Commun. 1989;163:520–526.

[9] Kitamura A, Nagasawa H, Kataoka; Ando T, Suzuki A. Amino acid sequence of pheromone-biosynthesis-activating neuropeptide-II (PBAN-II) of the silkworm, *Bombyx mori*. Agric. Biol. Chem. 1990;54:2495–2497.

[10] Masler EP, Raina AK, Wagner RM, Kochansky JP. Isolation and identification of a pheromonotropic neuropeptide from the brain-subesophageal ganglion complex of *Lymantria dispar*: A new member of the PBAN family. Insect Biochem. Molec. Biol. 1994;24:829–836.

[11] Matsumoto S, Kitamura A, Nagasawa H, Kataoka H, Orikasa C, Mitsui T, Suzuki A. Functional diversity of a neurohormone produced by the subesophageal ganglion: Molecular identity of melanization and reddish coloration hormone and biosynthesis activating neuropeptide. J. Insect Physiol. 1990; 36:427–432.

[12] Nachman RJ, Kuniyoshi H, Roberts V, Holman GM, Suzuki A. Active conformation of the pyrokinin/PBAN neuropeptide family for pheromone biosynthesis in the silkworm. Biochem. Biophys. Res. Commun. 1993;193:661–666.

[13] Nachman RJ, Roberts VA, Dyson HJ, Holman GM, Tainer JA. Active conformation of an insect neuropeptide family. Proc. Natl. Acad. Sci. USA 1991;88:4518–4522.

[14] Nachman RJ, Roberts VA, Lange AB, Orchard I, Holman GM, Teal PEA. Active conformation and mimetic analog development for the pyrokinin/PBAN/diapause/pupariation and myosuppressin insect neuropeptide families. In: Hedin PA, Hollingsworth RM, Masler EP, Miyamoto J, Tompson DG, Eds. Phytochemicals for pest control. Am Chem Soc. Symposium Series 658. Washington, DC: American Chemical Society; 1997:277–291.

[15] Nachman RJ, Teal PEA. Amphiphylic mimics of pyrokinin/PBAN neuropeptides that induce prolonged pheromonotropic activity following topical application to a moth. In: Konopinsla D, Goldsworthy G, Nachman R, Orchard I, Rosinski G, Eds. Insects: Chemical, physiological and environmental aspects. Wroctaw University Press; 1998 in press.

[16] Nachman RJ, Teal PEA, Radel PA, Holman GM, Abernathy R. Potent pheromonotropic/myotropic activity of a carboranyl pseudotetrapeptide analog of the insect pyrokinin/PBAN neuropeptide family administered via injection or topical application. Peptides 1996;17:747–752.
[17] Raina AK, Jaffe H, Kempe TG, Keim P, Blocker RW, Falcon HM, Riley CT, Klun JA, Ridgway RL, Hayes DK. Identification of a neuropeptide hormone that regulates sex pheromone production in female moths. Science 1989;244:796–798.
[18] Schoofs L, Holman GM, Hayes TK, Nachman RJ, De Loof A. Isolation, primary structure, and synthesis of locustapyrokinin: A myotropic peptide of *Lucusta migratoria.* Gen. Comp. Endocrinol. 1991;81:97–104.
[19] Schoofs L, Holman GM, Hayes TK, Nachman RJ, De Loof A. Structure, function and distribution of insect myotropic peptides. In: Davey KG, Peter RE, Tobe SS, Eds. Perspectives in comparative endocrinology. Ottawa: National Research Council of Canada; 1994:155–165.
[20] Teal PEA, Nachman RJ. Pheromonotropic activity of pseudopeptide mimics of insect pyrokinin neuropeptides after topical application or injection into a moth. Regulatory Peptides 1998;72:161–167.